Nitrate Reduction

Many chemorganoheterotrophs (bacteria that require organic compounds as their sources of energy, electrons and carbon) can conduct respiration and use nitrate (NO_3^-) as the final electron acceptor. This is a type of anaerobic respiration because something other than O_2 serves the final electron acceptor at the end of the respiratory electron transport chain. (If O_2 serves the final electron acceptor at the end of the respiratory electron transport chain, the organism is conducting aerobic respiration). [Note that anaerobic respiration is totally different from fermentation reactions. Fermentation reactions can also occur in the absence of oxygen, but a respiratory electron transport chain is not involved during fermentation.]

When nitrate (NO_3^-) is used as the final electron acceptor during anaerobic respiration it is reduced (gains electrons) to nitrite (NO_2^-) by nitrate reductase (Fig. 6-64, page 68-69 of the Atlas). Some of these bacteria possess the enzymes to further reduce the nitrite to either the ammonium ion or molecular nitrogen, N_2 or other gaseous forms of nitrogen such nitric oxide (NO) or nitrous oxide (N_2O), (Fig. 6-64, page 68-69 of the Atlas). The reduction of nitrate via anaerobic respiration to gaseous forms of nitrogen (N_2, N_2O or NO) is called denitrification

The ability of some bacteria to reduce nitrate can be used in their identification. For example *Escherichia coli* can reduce nitrate (NO_3^-) only to nitrite (NO_2^-), and no further. *Pseudomonas fluorescens* reduces nitrate completely to molecular nitrogen (N_2). *Staphylococcus epidermidis* is unable to use nitrate as a terminal electron acceptor for respiration

The nitrate reductions test is performed by growing bacteria in a culture tube with a nitrate broth medium containing 0.5% potassium nitrate (KNO_3). Each tube also contains an inverted Durham tube to collect any gas that might be formed. After incubation, the culture is examined for the presence of gas and nitrite ions in the medium. The gas is a mixture of CO_2 from respiration of energy-rich organic compounds via the citric acid cycle and N_2 released from the complete reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and then to molecular nitrogen (N_2). Nitrite ions can be detected by the addition of sulfanilic acid and N,N-dimethyl-1-naphthylamine to the culture. Any nitrite (NO_2^-) in the medium will react with these regents to produce a pink or red color (Fig. 6-65 and 6-67, p. 68-70 of the Atlas). Gas formation is determined by the presence of gas collected in the Durham tube.

If a culture does not produce a color change, several possibilities exist: (1) nitrate was not reduced to nitrite – there was no nitrate reduction; (2) the bacteria possess nitrate reductase and also further reduce the nitrite formed from that reaction to molecular nitrogen; (3) they possess other enzymes that reduce nitrite formed to ammonia. To determine if nitrates were reduced past nitrite, a small amount of zinc powder is added to the culture containing the sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents. Since zinc reduces nitrates to nitrites, a pink or red color will appear if nitrate was still present in the tube, that is, if nitrates were not reduced to nitrites by the bacteria (Fig. 6-68 on p. 70 of the Atlas). If a red color does not appear after addition of zinc powder (Fig. 6-68 on p. 70 of the Atlas), the nitrates in the medium were reduced past the nitrite stage to either ammonia or nitrogen gas. Ammonia (NH_3) is soluble in water as ammonium (NH_4^+) ions so ammonia would not contribute to gas bubble formation in the Durham tube. Nitrogen gas (N_2) is not very water-soluble and would accumulate in the Durham tube, forming a gas bubble. It is useful to add the sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents to an uninoculated control tube followed by the addition of zinc to see how zinc chemically reduces nitrate (NO_3^-, no red color) to nitrite (NO_2^-, red color will now form).

Simply scoring each tube either positive or negative can be somewhat confusing since various reagents are added sequentially. It is better to score the tubes as follows:

1. Nitrate was reduced to nitrite
(A red color formed when sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents were added to the tube, without addition of zinc powder)

2. Nitrate was reduced to nitrite and further reduced to N_2
(A red color did not form when sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents were added to the tube, and also *did not form* upon addition of zinc powder to the tube; a fairly large gas bubble collected in the Durham tube)

3. Nitrate was reduced to nitrite and further reduced to ammonia
(A red color did not form when sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents were added to the tube, and also *did not form* upon addition of zinc powder to the tube; no gas or only a small gas bubble (CO_2) collected in the Durham tube).

4. Nitrate was not reduced
(A red color did not form when sulfanilic acid and N,N-dimethyl-1-naphthylamine reagents were added to the tube, but a red color *did form* upon addition of zinc powder to the tube).

Results #1,2 or 3 indicate nitrate reduction. Such organisms can conduct anaerobic respiration using nitrate (and perhaps nitrite and nitric oxide as well) as the final electron acceptor(s) in a respiratory electron transport chain. [Remember, once again, that anaerobic respiration, which involves an electron transport chain, is totally different from fermentation. Fermentation reactions can occur in the absence of oxygen, but fermentation does not involve an electron transport chain.]

Result #4 indicates no nitrate reduction occurred. Such organisms cannot conduct anaerobic respiration using nitrate as a final electron acceptor.